Elevated Fibrinogen and Homocysteine Levels Enhance the Risk of Mortality in Patients From a High-Risk Preventive Cardiology Clinic

Monica Acevedo, Gregory L. Pearce, Kandice Kottke-Marchant, Dennis L. Sprecher

Abstract—Fibrinogen (Fib) plays an important role in platelet aggregation and thrombus formation, and homocysteine (tHcy) causes endothelial dysfunction and injury. Therefore, an interaction toward an enhanced risk of thrombotic events and consequent mortality might be expected in patients with both factors elevated. To determine whether patients exposed jointly to high Fib and high tHcy were at increased risk of mortality, we compared them with those with only one or neither risk factors elevated. Prevalence of coronary artery disease (cross-section) and short-term mortality (30:14 months) were assessed in 2084 patients with available baseline tHcy and Fib. Upper quartiles were used to define high tHcy (>14.2 μmol/L) and high Fib (>382 mg/dL). Cox models adjusting for Framingham risk score, creatinine, and coronary artery disease status were used to estimate the risk of high tHcy and high Fib and their combinations. Mean age of the patients was 56±12 years (35% women) with 71 (3.4%) recorded deaths. Risk-adjusted longitudinal models showed a hazard ratio of 2.14 (P=0.03) for isolated high tHcy, 2.28 (P=0.02) for isolated high Fib, and 3.29 (P<0.001) for both high tHcy and high Fib in comparison with neither risk factor high. Independence of each parameter and lack of synergism was found on longitudinal as well as cross-sectional analyses. Conjoint elevation of Fib and tHcy increased the risk of death by approximately 3-fold in three years. Although no significant interaction between Fib and tHcy was demonstrated, both provided independent information after adjustment for traditional risk factors. (Arterioscler Thromb Vasc Biol. 2002;22:1042-1045.)

Key Words: fibrinogen ■ homocysteine ■ mortality ■ risk

Fibrinogen (Fib) and homocysteine (tHcy) have both been reported as markers for cardiovascular (CV) clinical outcomes. Fib serves as a critical cross-link for glycoprotein IIb/IIIa on adjacent platelets and, therefore, represents a major step in platelet aggregation. Fib is also the main substrate for the coagulation cascade and forms a polymerized fibrin clot. tHcy, in contrast, impairs nitric oxide production and is implicated in the generation of oxidized species, leading to endothelial dysfunction and platelet activation. It has been implicated as a significant contributor toward mortality.

Because tHcy and Fib may play complementary roles in the platelet activation:aggregation cascade, it might be reasonable to suggest that a true interaction would be present in the clinical setting related to outcomes. Specifically, the outcome of total mortality would be markedly higher when both parameters were abnormally high than the addition of risks represented by each parameter individually.

We herein analyzed 3-year mortality in high-risk patients from a preventive clinic, with associated cross-sectional examination, to evaluate this potential interaction.

Methods

We selected all the patients (with and without coronary artery disease [CAD]) with a recorded tHcy and Fib, measured at the first consult to a Preventive Cardiology Clinic between January 1996 and March 2001 (n=2084). Any patients referred were included, with referral 80% from cardiology service and the rest predominately from internal medicine. We recorded the traditional CV risk factors including age, sex, body mass index, hypertension, diabetes, smoking, and family history of CAD. CAD was diagnosed in the presence of a documented acute coronary syndrome and/or stable angina, and/or a positive coronary angiography (stenosis >50% in ≥1 vessel), and/or CABG, and/or a positive noninvasive stress test. Self-reported CV disease history was partially validated via the institutional interventional registry and main clinical charts.

Laboratory Testing

Blood samples were drawn after a 12-hour fasting period at the first visit to the clinic. Assays performed included total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides, glucose, creatinine, and tHcy and Fib levels. Total fasting serum tHcy was measured by use of high-performance liquid chromatography as reported previously. The normal range in our laboratory is 3.92 to 17.12 μmol/L. Fib levels were measured in citrated plasma kept at −70°C by modification of the Clauss method (Dade Behring) with a Sysmex CA-6000 automated coagulation analyzer. The mean value in our laboratory is 300 mg/dL, with a reference range of 200 to 400 mg/dL.
Fibrinogen and homocysteine (tHcy) levels in the CAD population were measured an average of 37 months after the most recent qualifying cardiac event or procedure. Mortality data were obtained by searching the Social Security Administration database. We reported on total mortality in our study, believed to be an objective and unbiased endpoint.

### Statistics

Cox models were used to evaluate the relationships between mortality and tHcy and Fib. Upper quartiles for this population were used to define high tHcy (>14.2 μmol/L) and high Fib (>382 mg/dL). To test the hypothesis of an interaction between Fib and tHcy, patients were classified as having (1) neither high tHcy nor high Fib (≤335 μmol/L and ≤382 mg/dL respectively, n=1243), (2) isolated high tHcy (>14.2 μmol/L and ≤382 mg/dL Fib, n=320), (3) isolated high Fib (≥382 mg/dL and ≤14.2 μmol/L tHcy, n=335), or (4) both high tHcy and high Fib (>14.2 μmol/L and >382 mg/dL, n=186). Hazard ratios (HR) and 95% confidence intervals (CI) were calculated in unadjusted models and models adjusted for Framingham global risk score (including age, sex, LDL-C, HDL-C, systolic blood pressure, diastolic blood pressure, diabetes, and cigarette use).

Presence of known CAD and creatinine with separate modeling with individual covariates, age, sex, systolic blood pressure, total cholesterol, and CAD status were evaluated to corroborate the independent contribution of both tHcy and Fib. Group 1 served as the reference group for estimating mortality risk. Presence of CAD and history of myocardial infarction were evaluated on cross-section by using logistic regression to estimate the relative risk (using odds ratios [OR]) of groups 2, 3, and 4 in comparison with group 1 (with same model adjustments).

### Results

Baseline data are presented in Table 1. The mean follow-up interval was 30±14 months, and there were 71 (3.4%) deaths recorded.

Cad was reported in 1162 (56%) patients. Adjusted ORs for CAD determined from a cross-sectional analysis were (a) 1.54 (CI=1.18 to 2.01, P=0.001) for isolated high tHcy, (b) 1.35 (CI=1.05 to 1.74, P=0.02) for isolated high Fib, and (c) 1.73 (CI=1.19 to 2.52, P=0.004) for the combined presence of both high tHcy and high Fib. Similar results are seen for models specifically examining previous myocardial infarction (isolated high tHcy OR=1.59 [CI=1.21 to 2.08]; isolated high Fib OR=1.59 [CI=1.22 to 2.08]; high tHcy and high Fib OR=1.87 [CI=1.32 to 2.66]).

Fibrinogen and tHcy values were higher among the 71 deceased patients (median Fib 382 mg/dL vs 327 mg/dL and tHcy 14.6 μmol/L vs 11.3 μmol/L, P<0.001 for both). Mortality rates ranged from 1.5% (19 of 1243) for patients with neither high tHcy nor high Fib to 10.8% (20 of 186) for patients with both high tHcy and high Fib (Figure 1).

The unadjusted HR for mortality for the group of both high tHcy and high Fib was 5.63 (P<0.001) (Table 2). Lower but similar estimates of risk among the groups were produced after adjusting for Framingham scores, CAD status, and creatinine (Table 2). Individual covariates, eg, age, sex, total cholesterol, and systolic blood pressure, did not alter these outcomes. Further adjustments for aspirin and vitamin use also did not substantially change these results (eg, the conjoint elevation of tHcy and Fib produced a HR of 3.11 [CI=1.53 to 6.29] after adjustment for aspirin use and 3.27 [CI=1.63 to 6.58] after adjustment for vitamin use). Kaplan-Meier survival curves are shown for each group in Figure 2. Although the risk experienced by patients having both high tHcy and high Fib was elevated, there was no significant

### Table 1. Baseline Characteristics Overall and by Mortality Status at Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Alive at Last Follow-up</th>
<th>Deceased at Follow-up</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>2084</td>
<td>2013</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>720 (35%)</td>
<td>697 (35%)</td>
<td>23 (32%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Known CAD</td>
<td>1162 (56%)</td>
<td>1108 (55%)</td>
<td>54 (76%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>124 (94–158)</td>
<td>123 (93–157)</td>
<td>140 (110–176)</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>42 (35–52)</td>
<td>42 (35–52)</td>
<td>46 (35–52)</td>
<td>0.13</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129 (117–143)</td>
<td>129 (117–143)</td>
<td>138 (119–150)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79 (72–86)</td>
<td>79 (72–86)</td>
<td>89 (71–87)</td>
<td>0.86</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>205 (10%)</td>
<td>200 (10%)</td>
<td>5 (7%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Former</td>
<td>1007 (48%)</td>
<td>965 (53%)</td>
<td>42 (64%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diabetes</td>
<td>386 (19%)</td>
<td>364 (18%)</td>
<td>22 (31%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>1264 (61%)</td>
<td>1224 (61%)</td>
<td>40 (56%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Vitamin use</td>
<td>784 (38%)</td>
<td>767 (38%)</td>
<td>17 (24%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Framingham global risk score</td>
<td>6.0 (3.0–8.0)</td>
<td>6.0 (3.0–8.0)</td>
<td>7.0 (5.0–8.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>11.4 (9.2–14.2)</td>
<td>11.3 (9.1–14.1)</td>
<td>14.6 (11.9–19.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fib, mg/dL</td>
<td>330 (288–382)</td>
<td>327 (288–377)</td>
<td>382 (334–460)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.8–1.0)</td>
<td>1.0 (0.8–1.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continuous measures are presented as median and interquartile range, and categorical measures are shown as number and percentage with risk factor.
There was no evidence of a conjoint outcome beyond an additive effect ($P=0.42$).

To demonstrate independence for each of the two studied variables without any limits placed on the other, we also entered both into adjusted models for CAD (Fib quartile OR $=1.18$, CI $=1.09$ to 1.29, $P<0.001$; tHcy quartile OR $=1.35$, CI $=1.25$ to 1.47, $P<0.001$) and total mortality (Fib quartile HR $=1.54$, CI $=1.20$ to 1.97, $P<0.001$; tHcy quartile HR $=1.63$, CI $=1.27$ to 2.09, $P<0.001$).

### Discussion

In this longitudinal study, we have demonstrated that both elevated Fib and tHcy levels separately and independently predicted risk of death. No evidence for true synergism was found; however, the conjoint elevation of these 2 factors had a partially additive association for mortality, with an overall 3-fold increased risk (versus neither elevated) for 2 to 3 years.

Our hypothesis that the risk associated with concurrent elevations of Fib and tHcy would be greater than the addition of their respective individual risks could not be demonstrated. Although the coincident elevation in tHcy and Fib resulted in a 3-fold increased risk ($P<0.001$) suggesting greater risk than either that for Fib or tHcy alone, this is at best an additive effect, not synergistic. Similar results were observed on cross-sectional analyses to predict a history of myocardial infarction or CAD. The independent contribution of each factor (tHcy and Fib) to outcomes was observed both on cross-sectional and longitudinal evaluation, further implicating an additive influence. Although the number of deaths is limited, the HR associated with conjoint elevation of tHcy and Fib would have had to be approximately three times that for the isolated risks to show a statistically significant interaction.

Fib plays a key role as ligand for the platelet glycoprotein IIb/IIIa receptor. Such a pivotal role in platelet physiology also parallels the consistency of the results of a recent meta-analysis,$^{13}$ which showed an increased CV risk for elevated Fib levels. In contrast, tHcy is one of multiple factors which results in endothelial damage and activation of cells including platelets.$^{14}$ The results of prospective studies related to tHcy and CV prediction, however, have been controversial.$^2$

The prediction of synergism between these two markers could be based on at least two theories: a) the critical interdependence of platelet activation related to tHcy and aggregation related to Fib, and b) the tHcy-induced production of tissue factor which enhances vessel-wall adhesion of the platelet-glycoprotein IIb/IIIa complex.$^{14}$ The incremental elevation in HR when both high tHcy and high Fib were present beyond that when only one factor is high supports separate contributions to risk despite a reported direct Fib-induced platelet activation$^{15}$ and further implicates and strengthens the singular contribution of tHcy and other platelet-activating factors. The lack of a major aspirin influence on the interplay between these 2 factors, while maintaining their independent contributions, helps emphasize alternative non-thromboxane-associated pathways toward acute coronary events.$^{16}$

There are several limitations in the present study. 1) This was an observational study of a selected high-risk CV population. 2) Adjustments of the results were only made for baseline data and selected medications. 3) Finally, study-

### Table 2. Cox Regression Results for Mortality Models

<table>
<thead>
<tr>
<th>Group</th>
<th>Fib, mg/dL</th>
<th>tHcy, μmol/L</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>$\leq 382$</td>
<td>$\leq 14.2$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Group 2</td>
<td>$\leq 382$</td>
<td>$&gt; 14.2$</td>
<td>2.57 (1.37–5.01)</td>
<td>2.14 (1.09–4.20)</td>
</tr>
<tr>
<td>Group 3</td>
<td>$&gt; 382$</td>
<td>$\leq 14.2$</td>
<td>2.78 (1.43–5.41)</td>
<td>2.28 (1.15–4.50)</td>
</tr>
<tr>
<td>Group 4</td>
<td>$&gt; 382$</td>
<td>$&gt; 14.2$</td>
<td>5.63 (2.98–10.52)</td>
<td>3.29 (1.64–6.62)</td>
</tr>
</tbody>
</table>

HRs with 95% CIs and $P$ values are presented using patients with neither high tHcy nor high F (group 1) as the reference group.

Results presented as unadjusted and adjusted (Framingham global risk score, CAD, and creatinine).

There was no evidence of a synergistic interaction ($P=0.42$).
subject vital status was only assessed through the Social Security National Death Index. Therefore, no data on specific causes of death are available.

In summary, tHcy provides an additive, although not interactive, contribution to the well established future risk related to elevated fibrinogen levels. These data are consistent with the independent role of tHcy in augmenting thrombosis outside of a Fib-based mechanism. The significant predictive value of Fib and tHcy in our study when both factors are elevated (demonstrated in both cross-sectional and longitudinal evaluations) suggest value in appreciating the conjoint presence of these nontraditional variables in clinical practice.

References
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